Data Evaluation Report on the leaching of chlormequat chloride in aged soil columns

PMRA Submission Number {.....} EPA MRID Number 46715230

Data Requirement: PMRA Data Code:
EPA DP Barcode: D325184
OECD Data Point:
EPA Guideline: 163-1

Test material:

Common name: Chlormequat chloride.

Chemical name:

IUPAC name: 2-Chloroethyltrimethylammonium chloride.

CAS name: 2-Chloro-N,N,N-trimethylethanaminium chloride.

CAS No.: 999-81-5.

Synonyms Cycocel; Stabilan.

Smiles string: CN(C)(C)CCCl.[Cl-] (ISIS v2.3/Universal SMILES).
ClCCN(Cl)(C)(C)C (EPI Suite, v3.12).

Primary Reviewer: Kindra Bozicevich
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Date: 10/17/06

Company Code:
Active Code:
Use Site Category:
EPA PC Code: 018101

CITATION: Zohner, A. 1995. ^{14}C-Chlormequat chloride (^{14}C-CCC): aged residue leaching under laboratory conditions in one soil. Unpublished study performed by Agrolinz Melamin GmbH, former Agrolinz Agrarchemikalien GmbH, Leonding, Austria; sponsored and submitted by BASF Corporation, Research Triangle Park, NC. Report Numbers: M 94-27; 1231. BASF Registration Document Number: 1998/10587. Experiment initiation December 28, 1994 and completion February 7, 1995. Final report issued May 11, 1995.

EXECUTIVE SUMMARY

The column leaching of aged [CCC-^{14}C]-labeled 2-chloroethyltrimethyl ammonium chloride (chlormequat chloride; radiochemical purity >99%) was investigated in a loamy sand soil [pH 5.8, organic matter 1.3%] from Germany. Samples of the test soil were treated with [CCC-^{14}C]chlormequat chloride, representing a field rate of 1.38 kg a.i./ha, and connected to a series of traps containing 2N NaOH, ethylene glycol, and 2N H_2SO_4. Air was pumped through the samples (60 mL/minute) with a membrane pump. The soil samples were incubated at 20 ± 2°C for up to 15 days. Water loss determinations, analysis of the adsorption trapping solutions, and replacement of the sodium hydroxide solutions were performed once a week. For leaching, two glass columns (40-cm length; 5.0-in i.d.) were packed with 28 cm of sieved, untreated test soil and fully saturated with 0.01M $CaCl_2$ solution. Aged, treated test soil was then added to the surface of each packed soil column, and the columns were leached with four portions of 0.01M $CaCl_2$ solution added over a 48-hour period (temperature and lighting conditions not reported). Leachates were collected in four fractions. Soil from the soil columns was not analyzed or characterized.

Following application of [CCC-^{14}C]chlormequat chloride, the soil samples were extracted twice with methanol, three times with methanol:water, and five times with water pH 2. Following 15 days of aging, soil samples were extracted four times with methanol:water and four times with water pH 2. The extracts and pooled extracts were analyzed for total radioactivity using LSC. The pooled extracts were concentrated by evaporation and analyzed using LSC analysis. The extracts were analyzed for [CCC-^{14}C]chlormequat chloride and its transformation products using TLC analysis. The remaining soil was air-dried, homogenized, and analyzed using LSC following combustion. Following aging, the trapping solutions were analyzed for total radioactivity using LSC. To confirm the presence of $^{14}CO_2$, the NaOH trapping solution was combined with barium chloride and the supernatant was analyzed using LSC. The samples were then connected to a gas washing flask containing 2N NaOH solution and the barium carbonate was decomposed by adding 2N H_2SO_4 through the air inlet. The trapping solutions were analyzed using LSC. The leachate fractions were analyzed using LSC.

The incubation temperature during aging was reported to be 20 ± 2°C; no supporting information was provided. The incubation temperature during leaching was not reported. Based on TLC analysis of the pooled extracts following application of the test material and during and following aging, [^{14}C]chlormequat chloride accounted for the majority of the radioactivity (not quantified) in the soil. Unidentified radioactivity comprised <10% of the applied radioactivity, and chromatographed near the origin of the chromatogram.

Following 15 days of aging, the mass balance was 97.53-105.23% of the applied radioactivity. Extractable and nonextractable residues accounted for 46.08-49.20% and 17.68-23.25% of the applied, respectively. Cumulative [^{14}C]CO_2 comprised 32.76-33.76% of the applied, collected on days 7 and 15 of aging. Radioactivity recovered from the ethylene glycol and H_2SO_4 trapping solutions was ≤0.01% of the applied. The soil biomass at the beginning and end of the aging period was 2.37 mg CO_2/h/100 g dry weight and 2.02 mg CO_2/h/100 g dry weight, respectively.

A mass balance following the 2-day leaching period was not determined. The pooled leachates contained 0.29-0.49% of the applied radioactivity. Data characterizing the radioactivity in the soil columns were not provided.

Study Acceptability: This study is classified as **supplemental**. No significant deviations from good scientific practices were noted. The radioactivity remaining in the soil columns following leaching was not analyzed or characterized, so that a pattern of leaching for chlormequat chloride was not clearly established.

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: This study was conducted in accordance with Biologische Bundesanstalt Deutschland (BBA); FAO, Revised Guidelines on Environmental Criteria for the Registration of Pesticides (Dec. 1989); and Annex II, 91/414/EEC Directive (Jan. 1993; p. 13). The following significant deviations from the objectives of Subdivision N guidelines were noted:

> Radioactive residues remaining in the soil columns following the leaching period were not analyzed or characterized.

COMPLIANCE: This study was conducted in compliance with USEPA FIFRA Good Laboratory Practice Standards Title 40 CFR Part 160, Austria GLP Regulations, and OECD Principles of GLP (pp. 3-4). Signed and dated No Data Confidentiality, GLP, Quality Assurance statements were provided (pp. 2-5). A Certificate of Authenticity was not provided.

A. MATERIALS:

1. Test Material [CCC-^{14}C]Chlormequat chloride (p. 14).

Chemical Structure: See DER Attachment 1.

Description: Technical grade.

Purity:

Radiolabeled:	Radiochemical purity: >99% (pp. 14, 22; Figures 2-3, pp. 39-40). Batch No. 94238. Specific activity: 555 MBq/mmol (15.00 mCi/mmol. Locations of the label: 1 and 2 carbons of the ethyl group.
Storage conditions of test chemicals:	Stored frozen in a sealed glass bottle (p. 14).

Physico-chemical properties of chlormequat chloride:

Parameter	Value	Comment
Molecular formula	Not reported.	
Molecular weight	158.07 g/mol.	
Water Solubility	Not reported.	
Vapor Pressure/Volatility	Not reported.	
UV Absorption	Not reported.	
Pka	Not reported.	
K_{ow}/log K_{ow}	Not reported.	
Stability of compound at room temperature, if provided	Not reported.	

Data were obtained from p. 14 of the study report.

2. Soil Characteristics

Table 1: Description of soil collection and storage.

Description	Loamy sand
Geographic location	Landwirtschaftliche Untersuchungs- und Forschungsanstalt, 6720 Speyer, Obere Langgasse 40.
Pesticide use history at the collection site	Not reported.
Collection procedures	Not reported.
Sampling depth (cm)	Not reported.
Storage conditions	Stored outdoors in the testing facility, in open concrete containers with natural growing weed since 1986.
Storage length	Soil was collected on the day of experiment initiation.
Soil preparation (eg: 2 mm sieved; air dried etc.)	Air dried, sieved (2 mm).

Data were obtained from pp. 20, 22 of the study report.

Table 2: Properties of the soil.

Property		**Speyer 2.1**
Soil texture [1]		Loamy sand
% Sand (>60 µ)		83.9
% Silt (2-60 µ)		15.0
% Clay (<2 µ)		1.1
pH ($CaCl_2$)		5.8
Organic carbon (%)		0.75
Organic matter (%) [2]		1.29
CEC (mval/100g)	Ca	2.62
	Mg	0.55
	K	0.21
	Na	0.02
Moisture at 1/3 atm (%)		Not reported.
Bulk density (g/cm^3)		Not reported.
Biomass (mg CO_2/h//100 g	Start of aging:	2.37
	End of aging:	2.02
Soil taxonomic classification		Not reported.
Soil mapping unit (for EPA)		Not reported.

Data were obtained from pp. 19, 36 of the study report.
1 Soil textural classification could not be confirmed (see Reviewer's Comment's).
2 Reviewer-calculated as % organic carbon × 1.72.

C. STUDY DESIGN:

1. Preliminary study: No preliminary studies were reported.

2. Definitive study experimental conditions: The mobility of [CCC-^{14}C]-labeled 2-chloroethyltrimethyl ammonium chloride (chlormequat chloride; radiochemical purity >99%; specific activity 555 MBq/mmol) was investigated in a loamy sand soil (pp. 14, 19).

Prior to study initiation, the test soil was air-dried and sieved (2 mm; pp. 20, 22). The soil was pre-incubated at room temperature for *ca.* 1 week at *ca.* 40% of maximum water capacity at conditions close to test conditions. Soil biomass determinations were conducted prior to and at the end of soil aging by four replicate measurements on aliquot samples of a control soil (pp. 19, 27).

For aging, the soil surfaces of six aliquots (109.19 g; 100 g dry weight) of moist loamy sand soil were treated with aliquots (5.0 mL) of a [CCC-^{14}C]chlormequat chloride application solution at a nominal application rate of 1.38 kg a.i./ha, assuming uniform distribution within a 5-cm soil layer and a soil density of 1.5 g/mL (pp. 22-23). An additional aliquot of loamy sand soil was

prepared to serve as a reserve sample. The treated soil samples were mixed by shaking and were stirred with a spatula. The spatula was rinsed with 0.01M $CaCl_2$ solution (0.6 mL) and the rinse was added to the soil samples. To determine the remaining radioactivity, the spatula was rinsed with methanol:water (1:1, v:v; 25 mL), and the radioactivity was subtracted from the dosage activity. Five of the treated samples and one control sample were connected to a series of adsorption traps containing 2N NaOH, ethylene glycol, and 2N H_2SO_4 (100 mL; p. 24; Attachments 1-3, pp. 53-55). A membrane pump was used to move air through the samples at a rate of 60 mL/minute. The soil samples were incubated at 20 ± 2°C for up to 15 days (pp. 9, 33). Water loss determinations, analysis of the adsorption trapping solutions, and replacement of the sodium hydroxide solutions were performed once a week.

The soil column leaching study was conducted by preparing two glass columns (40-cm length; 5.0-in i.d.) equipped with glass frits, glass wool, and quartz sand (pp. 18, 30-31; Attachment 4, p. 56). Each column was packed with untreated loamy sand soil (method not specified) to a height of 28 cm. The columns were fully saturated with 0.01M $CaCl_2$ solution overnight and the maximum water capacity was determined. Two samples of aged, treated test soil were transferred to the surface of the two packed soil columns via a spatula. The spatula was rinsed with methanol:water (1:1, v:v; 100 mL), and aliquots (2 × 1 mL) of the rinsate were analyzed for total radioactivity using LSC. Each column was leached with four portions of 0.01M $CaCl_2$ (393 mL) solution added over a 48-hour period (experimental temperature and lighting conditions not reported). Four fractions of leachate were collected in Erlenmeyer flasks.

3. Description of analytical procedures:

Extraction/clean up/concentration methods: Extraction methods were performed on soil samples following application of the test material, during the aging period, and at the end of the aging period (pp. 27-29).

Following application of [CCC-^{14}C]chlormequat chloride, a single soil sample was extracted by shaking with methanol (2 × 100 mL; Extracts 1-2), methanol:water (1:1, v:v; 3 × 100 mL; Extracts 3-5), and water pH 2 (HCl; 5 × 250 mL; Extracts 6-10) for 15 minutes each (pp. 27-28). Following each extraction, the samples were centrifuged, the extracts were removed via a pipette, and an aliquot (1 × 0.1 mL) was analyzed for total radioactivity using LSC. The extracts were combined, and aliquots (2 × 1 mL) were analyzed using LSC. Extracts 1-7 and 8-10 were pooled and analyzed using TLC.

Following 13 days of aging, a single soil sample was extracted by shaking with methanol:water (1:1, v:v; 4 × 100 mL; Extracts 1-4) and water pH 2 (HCl; 4 × 100 mL; Extracts 5-8) for 15 minutes each (pp. 21, 23; 28-29). Following each extraction, the samples were centrifuged and an aliquot (0.1 mL) was analyzed for total radioactivity using LSC. The extracts were combined and aliquots (2 × 1 mL) were analyzed using LSC. An aliquot (100 mL) of the pooled extract was concentrated to 34.4 mL by evaporation at 45°C and an aliquot (1 × 1.0 mL) was analyzed using LSC. Aliquots of the pooled extracts were also analyzed using TLC.

Following 15 days of aging, two soil samples were extracted by shaking with methanol:water (1:1, v:v; 4 × 250 mL; Extracts 1-4) and water pH 2 (HCl; 4 × 250 mL; Extracts 5-8) for 15 minutes each (pp. 29-30). Following each extraction, the samples were centrifuged and an aliquot (1 × 0.1 mL) was analyzed for total radioactivity using LSC. The extracts were combined and aliquots (2 × 1.0 mL) were analyzed using LSC. An aliquot (100 mL) of the pooled extract was concentrated to 33.5 mL or 37.3 mL by evaporation at 45°C. Aliquots of the pooled extracts were also analyzed using TLC.

Total ^{14}C measurement: Aliquots (1 × 0.1-1.0 mL) of the leachate fractions and extracts were analyzed for total radioactivity using LSC (p. 24). For the trapping solutions, an aliquot (1 × 5 mL) of the NaOH trapping solution was diluted with water (45 mL) and aliquots (2 × 0.5 mL) were analyzed using LSC (pp. 24, 26). Aliquots (1 × 0.5-1.0 mL) of the ethylene glycol and H_2SO_4 trapping solutions were analyzed using LSC. Material balances were determined by summing the radioactivity recovered from the extractables, non-extractables, and trapped volatiles (pp. 27, 29).

Non-extractable residues, if any: Following aging, the soil samples were air-dried, homogenized, and analyzed for total radioactivity using LSC following combustion; combustion efficiency was not reported (pp. 25, 29)

Determination of volatile residues: Following aging, to confirm the presence of $^{14}CO_2$, an aliquot (1 × 5 mL) of NaOH trapping solution was combined with barium chloride (1 g), and the resulting precipitate was removed by centrifugation (p. 26). Duplicate aliquots of the supernatant were analyzed for total radioactivity using LSC. The samples were then capped with an inlet-outlet tube for purging air and connected to a gas washing flask containing 2N NaOH solution (100 mL). The barium carbonate was decomposed by adding 2N H_2SO_4 (20 mL) through the air inlet. The evolved CO_2 was adsorbed by purging the samples with a gentle stream of air for 30 minutes. Aliquots of the trapping solutions were analyzed using LSC.

Derivatization method, if used: A derivatization method was not employed in this study.

Identification and quantification of parent compound: Following aging, aliquots of the extracts were analyzed for [^{14}C]chlormequat chloride using TLC on cellulose F plates (20 × 20 cm; 0.1 mm thickness) developed in n-butanol:acetic acid:water (4:1:5, v:v:v; LMSS1) and n-butanol:ethanol:acetic acid:water (8:2:1:3, v:v:v:v; LMSS3; p. 25). Following development, areas of radioactivity were detected and quantified using a TLC Linear Analyzer. Unlabeled reference compounds were visualized by spraying with "Dragendorff" reagent. Samples were co-chromatographed with an unlabeled reference compound of 2-chloroethyltrimethyl-ammonium chloride (chlormequat chloride; purity 99.3%; Batch No.: WH-129/A; Control No.: 9463257; p. 15). Rf values for unlabeled chlormequat chloride were 0.45 (LMSS1) and 0.38 (LMSS3).

Identification and quantification of transformation products, if appropriate: Samples were analyzed for transformation products of chlormequat chloride using the same method as described for the parent. Samples were co-chromatographed with an unlabeled reference compound of ([2-hydroxyethyl]-trimethylammonium)-chloride (cholinchloride; purity 99.0%; Catalog No.: 23.994-1; pp. 16, 26). Rf values for unlabeled cholinchloride were 0.30 (LMSS1) and 0.22 (LMSS3).

Detection limits (LOD, LOQ) for the parent compound: Limits of detection (LOD) and Limits of Quantification (LOQ) were not reported.

Detection limits (LOD, LOQ) for the transformation products, if appropriate: Limits of detection (LOD) and Limits of Quantification (LOQ) were not reported.

II. RESULTS AND DISCUSSION

A. TEST CONDITIONS: The incubation temperature during aging was reported to be 20 ± 2°C; no supporting information was provided (p. 24). The experimental temperature employed during the leaching period was not reported. Based on TLC analysis of the pooled extracts following application of the test material and during and following aging, [CCC-^{14}C]chlormequat chloride accounted for the majority of the radioactivity (not quantified) in the soil (p. 33; Figures 6-15, pp. 43-52). Unidentified radioactivity chromatographed near the origin of the chromatogram comprised <10% of the applied radioactivity. Soil biomass at the beginning and end of aging was 2.37 mg CO_2/h/100 g dry weight and 2.02 mg CO_2/h/100 g dry weight, respectively (pp. 19, 36).

B. MASS BALANCE: The mass balance following the 15-day aging period was 97.53-105.23% of the applied for the loamy sand test soil (p. 33). A mass balance following the 2-day leaching period was not determined.

C. LEACHING: Following the 15-day aging period, extractable and nonextractable residues comprised 46.08-49.20% and 17.68-23.25% of the applied, respectively. Cumulative [^{14}C]CO_2, measured on days 7 and 15, comprised 32.76-33.76% of the applied. Radioactivity recovered from the ethylene glycol and H_2SO_4 trapping solutions was ≤0.01% of the applied.

Following the 2-day leaching period, 0.29-0.49% of the applied radioactivity was recovered from the pooled leachates (p. 35). Radioactive residues remaining in the soil columns following leaching were not analyzed for or characterized.

III. STUDY DEFICIENCIES

Radioactive residues remaining in the soil columns following the leaching period were not analyzed or characterized.

IV. REVIEWER'S COMMENTS

1. The experimental temperature employed during the leaching period was not reported. The experimental temperature should be maintained within the range of normal environmental parameters (18-30°C).

2. The soil textural class assigned by the study author was uncertain because the particle size distribution used in soil characterization was not according to the USDA Soil Textural Classification System. Attachment 3 of the study report includes a guide for the textural classification of the loamy sand soil according to the US-Soil Taxonomy. In this guide, the particle size fraction 2-60 µm was arbitrarily set equivalent to a fraction of 2-50 µm.

3. The method used to maintain a constant column head during leaching of the soil columns was not reported.

4. The study author stated that the amount of $^{14}CO_2$ liberated (*ca.* 15-16% of the applied) indicated complete mineralization of the [^{14}C-CCC] molecule (p. 36). These results were reported to be n agreement with the results of an aerobic soil metabolism study (MRID 467152 submitted with this package), which shows comparable degradation of the test material.

5. The test soil was treated at a field rate of 1.38 kg a.i./ha (pp. 9, 22). The maximum recommended field application rate of chlormequat chloride was not reported. Subdivision N guidelines state that the test concentration should be roughly equivalent to the maximum proposed or registered field application rate of the parent compound.

V. REFERENCES

1. U.S. Environmental Protection Agency. 1982. Pesticide Assessment Guidelines, Subdivision N, Chemistry: Environmental Fate, Section 163-1. Mobility studies. Office of Pesticide and Toxic Substances, Washington, DC. EPA 540/9-82-021.

2. U.S. Environmental Protection Agency. 1989. FIFRA Accelerated Reregistration, Phase 3 Technical Guidance. Office of the Prevention, Pesticides, and Toxic Substances, Washington, DC. EPA 540/09-90-078.

3. U.S. Environmental Protection Agency. 1993. Pesticide Registration Rejection Rate Analysis - Environmental Fate. Office of the Prevention, Pesticides, and Toxic Substances, Washington, DC. EPA 738.

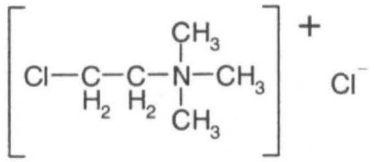
4. U.S. Environmental Protection Agency. 2003. Guidance for Calculating Sorption Coefficients in Batch Equilibrium Studies.

Attachment 1: Structures of Parent Compound and Transformation Products

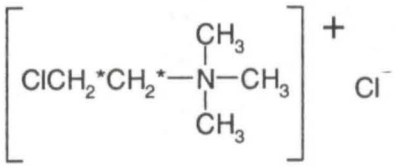
Chlormequat chloride [Cycocel]

IUPAC Name: 2-Chloroethyltrimethylammonium chloride.
CAS Name: 2-Chloro-N,N,N-trimethylethanaminium chloride.
CAS Number: 999-81-5.
SMILES String: CN(C)(C)CCCl.[Cl-] (ISIS v2.3/Universal SMILES).
ClCCN(Cl)(C)(C)C (EPI Suite, v3.12).

Unlabeled



[^{14}C]chlormequat chloride

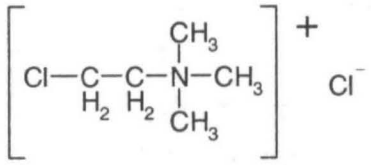


* = Position of radiolabel.

Identified Compounds

Chlormequat chloride [Cycocel]

IUPAC Name: 2-Chloroethyltrimethylammonium chloride.
CAS Name: 2-Chloro-N,N,N-trimethylethanaminium chloride.
CAS Number: 999-81-5.
SMILES String: CN(C)(C)CCCl.[Cl-] (ISIS v2.3/Universal SMILES).
ClCCN(Cl)(C)(C)C (EPI Suite, v3.12).



Unidentified Reference Compounds

Choline chloride

IUPAC Name: Not reported.
CAS Name: Not reported.
CAS Number: Not reported.